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Low Cost Material Enhanced the *in vitro* Regeneration and Micro Propagation of Medicinal Sand Dune Plant Species *Ipomoea Pes-caprae* (L.) R. Br.

P. Thirunavukkarasu, T. Ramanathan, G. Umamaheshwari, V. Manigandan and P. Dinesh

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, 608502, Tamil Nadu, India

Corresponding Author: P. Thirunavukkarasu, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai, 608502, Tamil Nadu, India Tel: 91+99952172249

ABSTRACT

Ipomoea pes-caprae is a sand dune plant commonly used as folklore medicine for fisherman communities based on the traditional knowledge. Little information is available on the feasibility of applying micropropagation techniques for production of dune and marsh species. The main aim of this study is to increase the callus induction and shoot generation of sand dune plant *Ipomoea pes-caprae* with easily available low cost natural materials. Coconut water is the rich source of carbohydrate and other nutrients which enhance callus and plant regeneration. In our present study, we tried different type of MS medium (Full and half strength) with coconut water at three different percentage (10, 15, 20%) and different concentration of plant growth regulators for callus induction and shoot regeneration. Well-developed callus inoculated in full and half strength MS medium with different concentration of CW and plant growth regulators. The best results were accomplished with half strength MS medium with 15% coconut water and with 2, 4-D and IAA 0.7 mg L^{-1} concentration which shows better callus induction and shoot regeneration. Young shoot and root developed plants transferred to green house and then followed to soil.

Key words: Sand dune, *Ipomoea pescaprae*, half strength MS medium, coconut water, callus culture, shoor regeneration

INTRODUCTION

Generally coastal sand dunes are formed by the sand deposited from sub tidal and intertidal regions. Wind is one of the most important factors, which help in formation, movements and distribution of sand dunes (Untawale and Nair, 1974). The sand dune vegetation has played a significant role in coastal region (Barson and Calder, 1981). It helps in prevention of sand erosion by decreasing wind speed at ground level. The sand dune vegetation is totally a different plant community with remarkable ability to locate hostile environment of drought, nutrient deficiency high winds, salts sprays and sand blast (Desai, 1995). *Ipomoea pes-caprae* (Convolvulacea) plant is highly reputed in folk and tribal medicines known by different names like railroad vine, coast morning glory, goat's-foot morning glory, salsa-da-praia etc., grows on sand dunes and beaches above the high tide line in tropical and subtropical regions throughout the world. *Ipomoea pes-caprae* has the potential in scavenging free radicals and can be a vital source of

antioxidant phytochemicals (Agoramoorthy *et al.*, 2008) and good antinociceptive property due to the presence of compounds, such as glochidone, betulinic acid, alpha- and beta-amyrin acetate, isoquercitrin in the writhing test and formalin test in mice and to treat dolorous processes (De Souza *et al.*, 2000).

Salinity is the major limiting factor influencing plant growth and productivity. High salinity and water scarcity cause ion imbalances, hyper-osmotic stress and ensuing secondary stresses (Li *et al.*, 2010). Halophytes have not been well studied for tissue culture due to heterozygosity and intricacy in establishing *in vitro* cultures (Uno *et al.*, 2009). Halophytes serve as model system for investigating adaptation mechanisms to saline environment. The sand dune plant of *Ipomoea pes-caprae is* used by coastal peoples as folklore medicine for many diseases. There is no evidence for *in vitro* regeneration of coastal medicinal plant of *Ipomoea pes-caprae*. The main aim of this study was to develop a micropropagation protocol for *Ipomoea pes-caprae*. Various plant culture mediums, plant growth regulators (PGRs) and low cost alternative natural materials were tried.

MATERIALS AND METHODS

Isolation of coconut water: The coconut water is simply drained from immature coconuts by drilling holes through two of the micropyles. Extract of water from each fruit separately was checked that it is not fermented before addition to the bulk. Collected water from all the fruits was heated at 60°C for 10 min with continuous stirring to precipitate out the proteins, fats and other materials.

Harvesting and sterilization of explants: Stem and leaves were excised from the healthy plant (*Ipomoea pes-caprae*) from the coastal and beach region of Parangipettai, Tamil Nadu, (South east coast of India). Explants were transported to the laboratory at 4°C. The explants were kept in running tap water for one hour and then washed with 0.5% bavestin for 3 min. Again explants were washed under tap water for 5 min followed by a 10 percent Tween 20 (Liquid detergent; Himedia, India) bath for 5 min, then the surface was sterilized with 70% (v/v) ethanol for 2 min and rinsed 3 times with sterile distilled water. Then surface-disinfected with 0.1% (w/v) aqueous mercuric chloride solution for 10 min and finally rinsed with autoclaved distilled water (five to seven times). The explants were grown in MS basal medium as well as in moistened cotton.

Culture conditions: *In vitro* raised explants segments of *Ipomoea pes-caprae* were cultured on Murashige and Skoog (1962) basal medium (MS) supplemented with 3% (w/v) sucrose (Himedia, India) and 0.8% (w/v) agar for culture initiation. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.8 with 1 N NaOH or 1 N HCl. In all the experiments, the chemicals used were of analytical grade (Himedia, Qualigens, Merck, Lobachemie and Fischerand Sigma). The medium was dispensed into culture vessels (Borosil, India) and autoclaved at 105 kPa (121°C) for 15 min. The explants were implanted on the culture medium (Conical flask of 250 mL volume containing 50 mL medium) and plugged tightly with non-absorbent cotton. All the cultures were incubated at 25±2°C under 16 h photoperiod of 45-50 μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes (Philips, India) and with 55-60% Relative Humidity (RH). All subsequent subcultures were performed at four week intervals.

Callus induction: The explants were cultured in different media full strength MS media (FMS), Half strength MS Media (HMS), Full strength MS media and coconut water (FMS+CW), Half

strength MS Media and coconut water (HMS+CW). The Coconut Water (CW) supplemented with three different percentage 20, 15 and 10% (v/v) combinations. The plant growth regulators such as 2,4-D, IAA, NAA and BAP added in different concentrations between 0.5-2.0 mg L^{-1}.

Plant regeneration medium: White friable calli were cultured on MS medium supplemented with Coconut Water (CW) with three different percentage 20, 15 and 10% (v/v) and with different concentrations of plant growth regulators, such as 2, 4 -D, IAA, NAA and BAP added in different concentrations between 0.5-2.0 mg L^{-1}. Out of the three mediums, the MS medium supplemented with 15% of coconut water was found to induce the maximum growth in *Ipomoea pes-caprae*. Hence, this medium was used throughout the experiment.

Rooting medium: Elongated shoots were excised from each culture passage and transferred to half strength concentration of MS medium supplemented with 15% of coconut water and different concentrations of IAA and NAA (0.5 -2.0 mg L^{-1}).

Acclimatization and transfer of plantlets to soil: Plantlets with well-developed roots were removed from the culture medium and after washing the roots gently under running tap water, plantlets were transferred to plastic pots (10 cm diameter) containing autoclaved garden soil, farmyard and sand (2:1:1). All were irrigated with 1/8 MS basal salt solution devoid of sucrose and inositol every 4 days for 2 weeks. The potted plantlets were covered with porous polyethylene sheets for maintaining high humidity and were maintained under the culture room conditions. The relative humidity was reduced gradually and after 30 days. The plantlets were transplanted to botanical evaluation garden and kept under shade in a nethouse for further growth and development.

Statistical analysis: Experiments were set up in a Randomized Block Design (RBD) and each experiment usually had 10 replicates and was repeated three times. Ten to fifteen explants were used per treatment in each replication. Observations were recorded on the percentage of response of callus formation, percentage of response of shoots, number of shoots per callus, shoot length, percentage of response of roots, roots per shoot and root length, respectively. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level according to Gomez and Gomez (1976).

RESULTS

Callus induction: The result revalued the induction of callus from *Ipomoea pes-caprae* in different types MS media with Coconut Water (CW). Full strength MS media composition (FMS) exhibited low callus induction in *Ipomoea pes-caprae* and also very poor growth. Half strength MS media exhibited 5% callus induction. But in the case of (FMS+CW); water, it is observed that 15% of growth and HMS+CW exhibited more than 59% of callus induction (Fig. 1, 2). In this study we compared different concentration of coconut water (10, 15 and 20%) for better results. Results showed 10% CW showed poor callus induction. The 20% CW observed in 30% of callus induction but 15% CW observed more than 59% of callus induction (Fig. 2). In case of plant growth regulators 2, 4-D and IAA 0.7 mg L^{-1} combination showed high level of callus induction (Fig. 3, 6a-b).

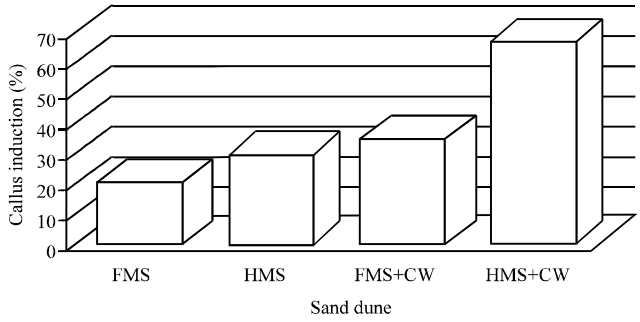

Fig. 1: Effects of normal and alternative MS medium to induction of callus of *Ipomoea pes-caprae*, Results are significant at ($p<0.05$) level, comparison by DMRT

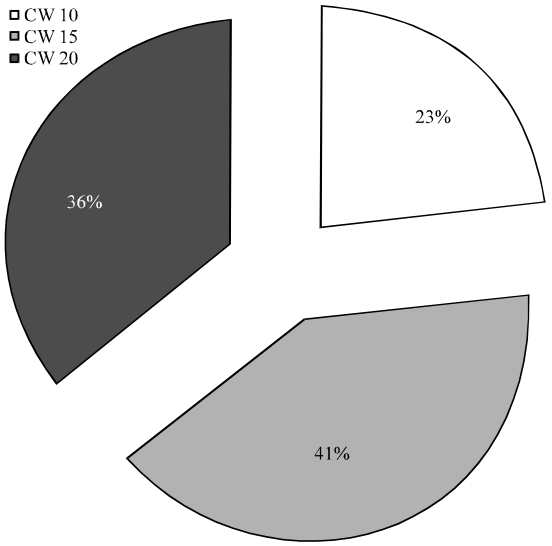

Fig. 2: Effects of coconut water to induction callus of sand dune species *Ipomoea pes-caprae*, Results are significant at ($p<0.05$) level, comparison by DMRT

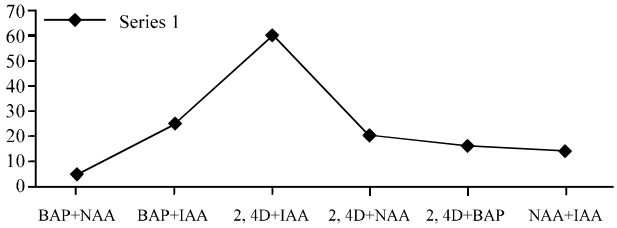

Fig. 3: Effect of plant growth regulators for callus induction of *Ipomoea pes-caprae*, Results are significant at ($p<0.05$) level, comparison by DMRT

Shoot regeneration: Well-developed callus from *Ipomoea pes-caprae* were cultured on MS with 15% of coconut water alone and with BAP, NAA, IAA for proliferation of sand dune plant species. The explants were found to be swollen and they produced 2-4 shoots within three weeks after

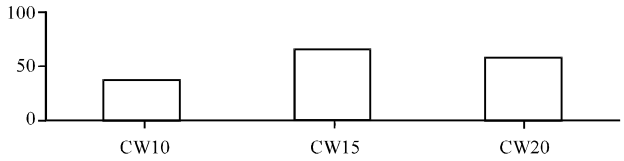

Fig. 4: Effects of coconut water to shoot regeneration of sand dune species-*Ipomoea pes-caprae*, Results are significant at ($p<0.05$) level, comparison by DMRT

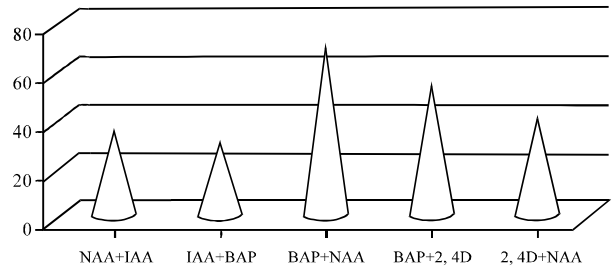

Fig. 5: Effects of plant growth regulators shoot regeneration of sand dune species -*Ipomoea pes-caprae*, Results are significant at ($p<0.05$) level, comparison by DMRT

inoculation on MS containing 15% CW alone but the number of shoots increased up to 9 when the explants were cultured in MS with 0.7 mg L^{-1} 2, 4D, BAP and NAA. All the combinations responded in the different strength medium (Full and Half) but highest number of micro-shoots was induced from CW at 15% with half strength (Fig. 4). The media without coconut water did not show proper growth of explants. Half strength MS medium with 15% of CW revolved that high level shoot regeneration in *Ipomoea pes-caprae* when compared to other concentrations. FMS, HMS and FMS+CW showed low level of shoot regeneration. The newly initiated shoots were separated and subcultured repeatedly in fresh MS+15% CW+0.7 mg L^{-1}+2, 4D, BAP and NAA, where the number of shoots increased up to 22.34±0.40 per culture (Fig. 5 and 6c). Root regeneration was highly observed in IAA in 0.9 µM concentration. Other combinations did not give good results (Fig. 6d).

DISCUSSION

Callus induction on MS medium containing various concentrations of plant growth regulators, but better growth observed in (2, 4D and IAA) at 0.7 mg L^{-1} with 15% CW in *Ipomoea pes-caprae* (Fig. 3). In our study it was observed that both CW, 2, 4D and IAA had a synergistic effect and either of them was not able to produce same result when used separately. Pelissier *et al.* (1990) undifferentiated callus from *Helianthus annuus* with respect to NAA, BA and coconut water Gill *et al.* (1993). The callus induction mostly observed in CW combination and very low level of callus induction observed in without CW combination (Fig. 2). Frequency of callus induction, type of callus and regeneration of plantlets were influenced on genotypes of *Ipomoea pes-fcaprae*. A similar result was reported by Patil and Kuruvinashetti (1998). The first subculture cycle had been very fast in the production of callus and it had high regeneration potential. The second and third subculture cycle of the callus has been brown in colour. The colour of the callus was pale yellow initially and also white colour callus was observed after 30 days of inoculation in all auxins and

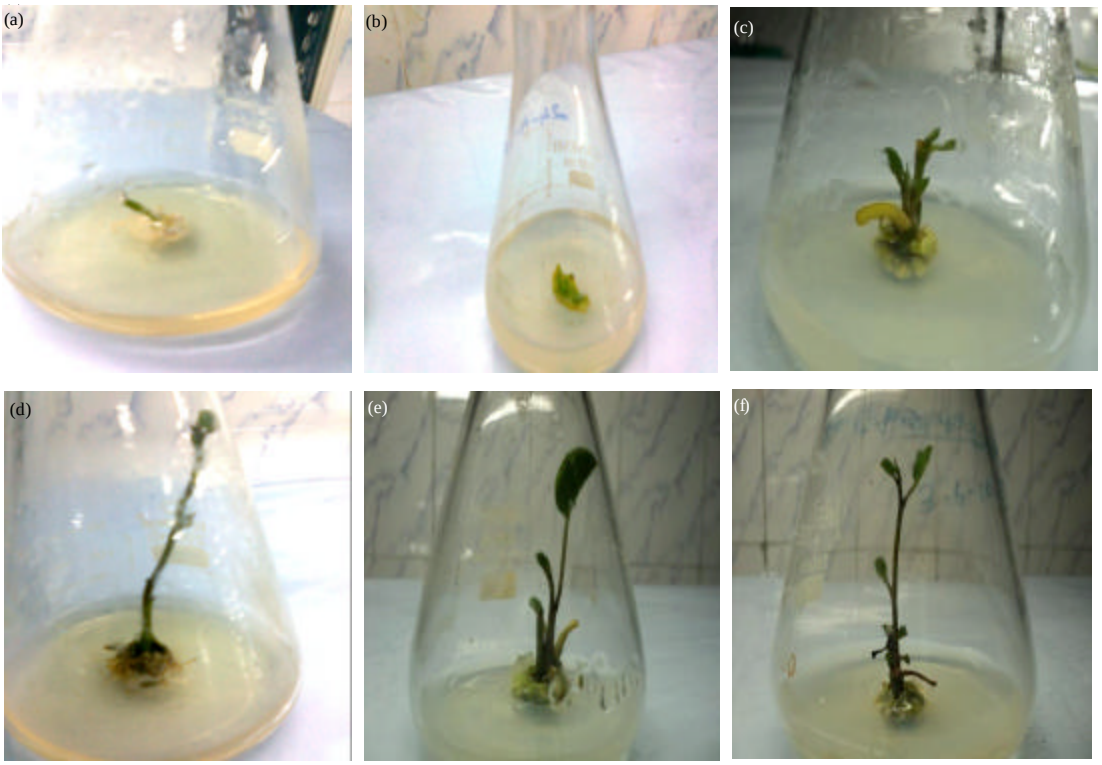

Fig. 6(a-f): Callus induction of *Ipomoea pes caprae* from, (a) Leaf explant (b) Stem, (c) Well developed callus to generate shoot, (d) Root developed from mature shoot, (e) Multi shoots from matured callus from 40 days and (f) Multi and branched shoots from matured callus 60 days culture

both varieties. Hagio (1994) reported that most of the varieties formed compact yellowish calli. In our results revolved 15% of CW and half strength of MS media high level of shoot regeneration (Fig. 1, 6c). The same result observed in previous study (Vartak and Shindikar, 2008), Mamun *et al.* (2004) reported that 2, 4-D and 10% coconut milk produced maximum amount of regenerative callus from leaf sheath in sugarcane. Callus can also be grown on medium without coconut water but at a much slower rate. Similar results were observed by Masteller and Holden (1970) in sorghum. The high frequency of callus was observed in HMS+CW (Fig. 1, 6a).

The shoot regeneration of *Ipomoeapes-caprae* in well developed callus with different concentration of CW and plant growth hormones. The results revolved that half strength of MS media with 15% of cw higher level of growth compare to others (Fig. 5, 6c). *In vitro* sprouling of mangrove plant species *Bruguira cylindrical* shows better growth in half strength of MS medium when compared to full strength medium. *Ipomoea pes-caprae*, it was also observed that multiple shoots were found by using different concentrations of cytokinin with auxins by other researchers (Faisal *et al.*, 2005; Gawde and Paratkar, 2004; Baskaran and Jayabalan, 2005; Hassan and Roy, 2005; Gopalakrishnan *et al.*, 2009; Hassan and Khatun, 2010; Ugraiah *et al.*, 2010). Among all the treatments in the combination of BAP, 2, 4D and NAA shoot proliferation is gradually increased with BAP and 15% CW. However, BAP+IAA and NAA combination (20 treatments) did not show significant shoot proliferation and most of the cultures died after 40days (Fig. 6e-f). Among

the various concentrations of auxins, the highest percentage of white friable callus was induced in the MS medium containing 9 μM 2, 4-D with 10% CW (Fig. 6a). Arti *et al.* (1994), Nguyen *et al.* (1998) and Saradamani *et al.* (2003) reported that higher levels of 2, 4-D or 2, 4, 5-T increased callus production. MS media supplemented with 2 mg L^{-1} BA and 2 mg L^{-1} IAA responded best for plant regeneration of cotton (Tripathy and Reddy, 2002). Root regeneration observed only in IAA plant growth promoter. The previous study showed same results as follow: The shoots developed from *Salvia canariensis* explants formed roots when transferred to half strength MS medium supplemented with IAA, NAA or IBA. Shoots were rooted most effectively in 1/2 MS medium supplemented with 1.0 mg L^{-1} IBA (Mederos-Molina, 2004). The good result observed in our present study, 1/2 MS strength of MS media enhanced the shoot and root formation in *Ipomoea pes-caprae* (Fig. 6d).

CONCLUSION

Sand dune plants differ from terrestrial mangrove plants. Generally salt tolerance or salt accumulated plants are very difficult for *In vitro* regeneration. The addition of coconut water to the culture media resulted in the plants with a greater nutritional and carbohydrate contents as coconut water itself contained 21.8 g L^{-1} sugars in total. In our present study, the effect of coconut water for the enhanced *in vitro* propagation of *Ipomoea pes-caprae* was evaluated. MS media containing 15% coconut water (v/v) with of 2, 4D and IAA0.7 mg L^{-1} combination resulted in better callus induction and the maximum increase in number and length of shoots. So, we concluded this kind of concentrations will be optimum for sand dune plants especially for *Ipomoea pes-caprae*.

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